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EFFECT OF DISSOLVED ORGANIC SUBSTANCES ON OYSTERS

BY ALBERT COLLIER, S. M. RAY, A. W. MAGNITZKY
AND JOE O. BELL

FISHERY BULLETIN 84

UNITED STATES DEPARTMENT OF THE INTERIOR, Douglas McKay, *Secretary*
FISH AND WILDLIFE SERVICE, John L. Farley, *Director*

ABSTRACT

Sea water contains unknown substances in minute quantities which respond to the N-ethyl-carbazole test for carbohydrates. Pumping rates and shell gapes of several oysters recorded simultaneously indicated a parallel reaction to the concentration of carbohydrates in sea water. The oysters responded to increases in the carbohydrate concentration by pumping increasing amounts of water, and exhibited thresholds of lower limits below which they did not pump. Higher water temperatures elevated this threshold.

Analysis of shell movements revealed three phases of gape: Phase I, preliminary and equal to about one-third total gape; phase II, transitional; and phase III, induced only by a carbohydrate concentration above the threshold value.

Study of the behavior of the substances in sea water stored under controlled conditions established that light and air increase their production, and that they remain constant in the dark and in filtered water. There is an inverse, noncausal relation to salinity in natural estuarial water.

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EFFECT OF DISSOLVED ORGANIC SUBSTANCES ON OYSTERS

By ALBERT COLLIER and S. M. RAY, *Fishery Research Biologists*, A. W. MAGNITZKY, *Oceanographer*, and JOE O. BELL, *Fishery Research Biologist*

the course of prolonged and detailed studies he effects of industrial wastes on oysters, *sostrea virginica* (Gmelin), continuous record- of the activities of several oysters were made ltaneously. We noted that the oysters tended ehave in a parallel fashion which could not be elated with any of the factors customarily sured. The work reported here presents our ts to define the cause or causes. A prelimi- paper on this subject (Collier, Ray, and nitzky, 1950), has demonstrated that an or- substance responding to the test for carbo- ates is associated with the activity of oysters. here is a large body of information concerning effect on oysters of those environmental factors h can be readily measured, including temper- e, salinity, pH, turbidity, and oxygen content. l recently, however, there have been no ana- al estimates of continuous variations in the nic content of sea water and their relation to activity of oysters. We were fortunate in ng at our disposal a technique for estimating in elements of the organic materials which to the test for carbohydrates. J. Gordon nan suggested the method used here and ted it to field conditions. The technique is ribed in the Appendix, page 182. It is impor- to note that all carbohydrate values are given erms of arabinose equivalents, and do not ssarily reflect actual concentrations of carbo- ate substances.

is our intent not to enter the argument con- ng the utilization of dissolved organic mate- by marine animals—an argument not yet d (Korringa 1949)—but only to demonstrate relation between the dissolved carbohydrates a water and the activity of oysters. This ion is discussed in the light of previous works on oyster physiology, together with some practical and theoretical implications.

TECHNIQUES

RECORDING THE ACTIVITY OF OYSTERS

We chose the simultaneous recordings of shell movements and pumping rates as the best available index of physiological activity, since both lend themselves to uninterrupted recording over long periods of time. The degree of shell gape alone could not be used, because gape is only the prerequisite to the filtration of water, and as long as it exceeds a critical point, the flow of water through the gills may vary considerably. The rate of removal of artificially introduced suspended materials gives a useful index of activity for short periods, for it can be measured quickly and apparently without affecting the oyster significantly. The method is not suitable for long-term studies, however, because it does not lend itself to the continued automatic recording of the oyster's activity in such a way that maximum detail of behavior is discernible in the record at any moment. Also, the carmine-cone and drop-counting techniques (Galtsoff 1928a and 1928b, Galtsoff et al. 1935), have limited value for this type of extended experiment not only because they can be used only for short-term observations, but also because they may seriously interfere with the neurosensory network controlling the water flow through the body of the oyster. In the carmine-cone method, the accumulation of carmine cannot be easily controlled, and its effect on the oyster cannot be measured as the experiment progresses.

While the pumping rate may not necessarily indicate feeding rate, this is not a legitimate objection to the use of the pumping rate as an index of physiological activity. Because the oyster takes its food from the water, there can be no doubt that the more water it passes through its filtering system, the more food it can get.

—Albert Collier, S. M. Ray, and Joe O. Bell, United States Fish ldlife Service; A. W. Magnitzky, United States Navy Hydrographic

Realizing that the pumping rate is a resultant of all the factors that influence the oyster, we accept it as the best available measure of physiological activity. The method used in measuring the pumping rate is described in the Appendix, page 183.

COLLECTING WATER SAMPLES

Sea water was pumped from the bay and supplied to the laboratory at Pensacola, Fla., as a continuous flow without storage, because it was desired to follow the changes in the bay water as they occurred.

During the course of the work, it became apparent that water samples were required at frequent intervals, owing to the wide and frequent variations in salinity and carbohydrate concentration noted in our preliminary tests. In September 1948 we began taking samples every 2 hours throughout the day and night, and in November 1949, the interval was shortened to 1 hour. The samples were collected by means of a special device described in the Appendix (fig. A–2).

CHARACTERISTICS OF CARBOHYDRATES

STABILITY

Since the effects of storage on these organic compounds, which we have defined as carbohydrates, were not known, it was necessary to determine their stability while in storage so as to establish the validity of values from samples which had been collected in the automatic sampler. We assumed that no significant change would occur during the intervals between analyses, and tested this assumption by collecting a series of triplicate samples from the laboratory sea-water supply (table 1). In each series, one was analyzed immediately, one was held overnight at room temperature, and one was held overnight in the refrigerator. The mean difference between the samples analyzed immediately and those held in the refrigerator was plus 0.007 milligram/liter, and for those held at room temperature was plus 0.094 milligram/liter. Since even the larger of these differences was within the range of error of our readings, we accepted this method of sampling as adequate, despite the delays inherent in making the determinations.

TABLE 1.—*Effect of standing time on carbohydrate centration (mg./l.) of sea water at Pensacola, Fla., 19*

Date	Hour	Immediate determination	Overnight in refrigerator	Difference	Overnight at room temperature	D e
May 10	1200	15.4	15.5	+0.1	15.5	
	1400	15.5	15.4	−.1	15.4	
11	1200	11.3	11.3	.0	11.2	
	1400	12.6	12.8	+.2	12.8	
16	1000	14.4	14.4	.0	14.4	
	1200	15.0	14.7	−.3	15.2	
17	1000	16 3	16.3	.0	16 3	
	1200	16.3	16.3	.0	16.8	
	1400	16.8	16.8	.0	17.0	
18	1000	8.6	8.6	.0	8.7	
	1200	5.8	6.0	+.2	6.0	
	1400	6.6	6.6	.0	6.8	
19	1000	6.3	6.1	−.2	6.3	
	1200	9.9	9.9	.0	9.9	
	1400	10.0	10.2	+.2	10.2	
21	0800	10.7	----------	----------	10.7	
	1000	11.5	----------	----------	11.5	
	1200	11.2	----------	----------	11.2	
Mean	----------	----------	----------	+.007	----------	

EFFECTS OF FILTERING AND CENTRIFUG

Since the carbohydrate content of diatom other organisms present in varying number particulate matter might cause erratic result the carbohydrate analysis, we tested the ma tude of this factor, by making a series of test filtered and on centrifuged sea water, separately and in combination. The result these tests are shown below.

Carbohydrate concentrations of three sam before and after certrifuging [1] showed the follo changes:

	Before	*After*	*L*
Sample 1	10. 7	10. 5	
Sample 2	13. 9	13. 7	
Sample 3	13. 9	13. 9	

Carbohydrate concentration of four sam before and after filtering [2] showed the follo changes:

	Before	*After*	*L*
Sample 1	11. 5	11. 3	
Sample 2	11. 2	11. 2	
Sample 3	13. 9	13. 6	
Sample 4	14. 1	13. 9	

From these results it became apparent each method was effective in removing partic matter. A sample containing 10.8 mg. of c hydrates to the liter was then centrifuged found to contain 10.5 mg. to the liter. An ali of that same sample, when filtered, containe

[1] Centrifuged for 10 minutes at force 336. See Appendix, p. 182.

[2] Zeitz filter, porosity permitting 1.5 liters per hour at 40 mill vacuum.

tical amount, 10.5 mg./liter. The filtered sam- when centrifuged, showed no change. These riments having demonstrated that either the inute centrifuging or the filtering was suffi- to remove the particulate matter which might erratic results, we adopted centrifuging as a dard part of the technique.

VARIATIONS IN CARBOHYDRATE NCENTRATIONS IN STANDING SEA WATER

e assumed that sea water with carbohydrates lved in it would also contain the organisms ssary for their production. Further, if the water were kept in jars in the laboratory, nges in species composition and in the numbers rganisms in it would be reflected in varying entrations of the carbohydrates. If these pounds were of biotic origin, they would be cted to increase more rapidly with aeration without; and if the producers were phyto- kton, production would be inhibited in the nce of light. In any case, the production of ohydrates would not be expected if the ative organisms were removed by filtering. rdingly, a set of experiments was designed to the conditions for growth and, by inference, blish which group of organisms was re- sible for the production of the carbohydrates. experiment A we filled duplicate jars with sea water. One was supplied with air by ns of a small aquarium pump connected at bottom to a sintered glass block; the other left undisturbed. Samples were taken from center of the jars by siphons, so calibrated the water standing in them could be meas- and discarded at each sampling. These were kept about 15 feet from the west windows he laboratory and no lights were kept on at t, an arrangement which was followed with the cated modifications in the other experiments he series. In this experiment it was evident the production of carbohydrates was ulated by aeration (fig. 1–A).

gitation, as produced by the aerating appa- s, might have stimulated multiplication of micro-organisms, and so account for the dif- nces indicated in experiment A. To test this set up experiment B, in which the surface r was gently ventilated without agitation. ples were taken near the bottom of the jar and from the surface of the water. The results show that the increased production of carbohydrates in the aerated water of experiment A was not due to agitation (fig. 1–B), but to the increased aeration. Here the carbohydrate concentration increased more rapidly and reached a higher concentration at the surface. It appears that part of the belated increase in the bottom concentration might have been the result of mixing due to convection currents in the jar.

In experiment C, filtered water was used to determine the sequence of changes when it could be assumed that the organisms which produce the substances had been removed from the water. As in experiment A, one jar was aerated and one was undisturbed. It was quite evident from the low carbohydrate concentration that the organisms producing these substances had been removed by filtration (fig. 1–C).

Experiment D was designed to demonstrate the role of light in the production of carbohydrates. The sequence of changes in both filtered and unfiltered sea water kept in the dark was followed. No significant changes in carbohydrate concentrations occurred in the absence of light (fig. 1–D).

From these experiments we may conclude that biotic activity is responsible for the production of carbohydrates, and that light and air create conditions favorable for the organisms producing these carbohydrates in sea water. It would be expected further that the responsible organisms are in part, if not altogether, photosynthetic, as indicated by the increased production in the presence of light. The effect of aeration is not so conclusive, for it is not known whether the air supplied carbon dioxide to plants, or oxygen to heterotrophic organisms (dinoflagellates).

RELATION OF CARBOHYDRATE CONCENTRATION TO SALINITY

We tabulated and averaged the carbohydrate-concentration values falling within salinity ranges of 2°/₀₀. Plotting of these averages shows an association between the concentration of carbohydrates and salinity (fig. 2). Between about 15°/₀₀ and 31°/₀₀ there is an apparent negative correlation between salinity and carbohydrates. From 7°/₀₀ to about 15°/₀₀ this relation is not clear and may not exist. The negative relation at the higher salinities may have been caused by the invasion

of the carbohydrate-poor, high-salinity waters of the Gulf of Mexico into the carbohydrate-rich and low-salinity waters of Santa Rosa Sound. Whether this means that the correlation is due to the falling off in the carbohydrate values in proportion to the volume of gulf water intruding, or reflects an inverse relation between carbohydrate production and salinity in gulf waters, is not evident in these data. Lack of knowledge o origin of these carbohydrates precludes spe tion on the part that might be played by salinity tolerance of the organisms responsibl their production. The association of carb drates with salinity illustrated could be the r of both physical dilution and biological i ference due to salinity changes.

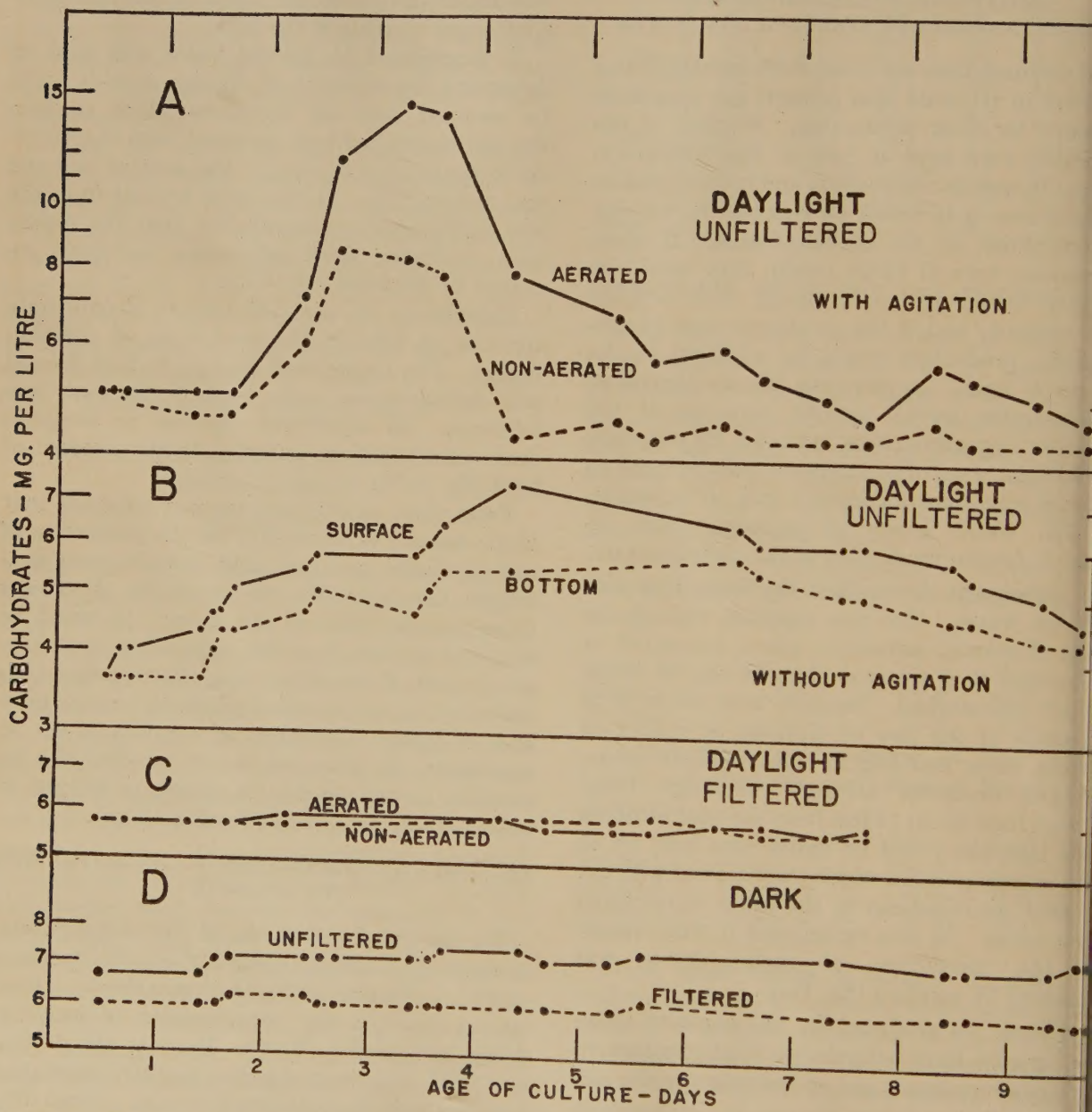
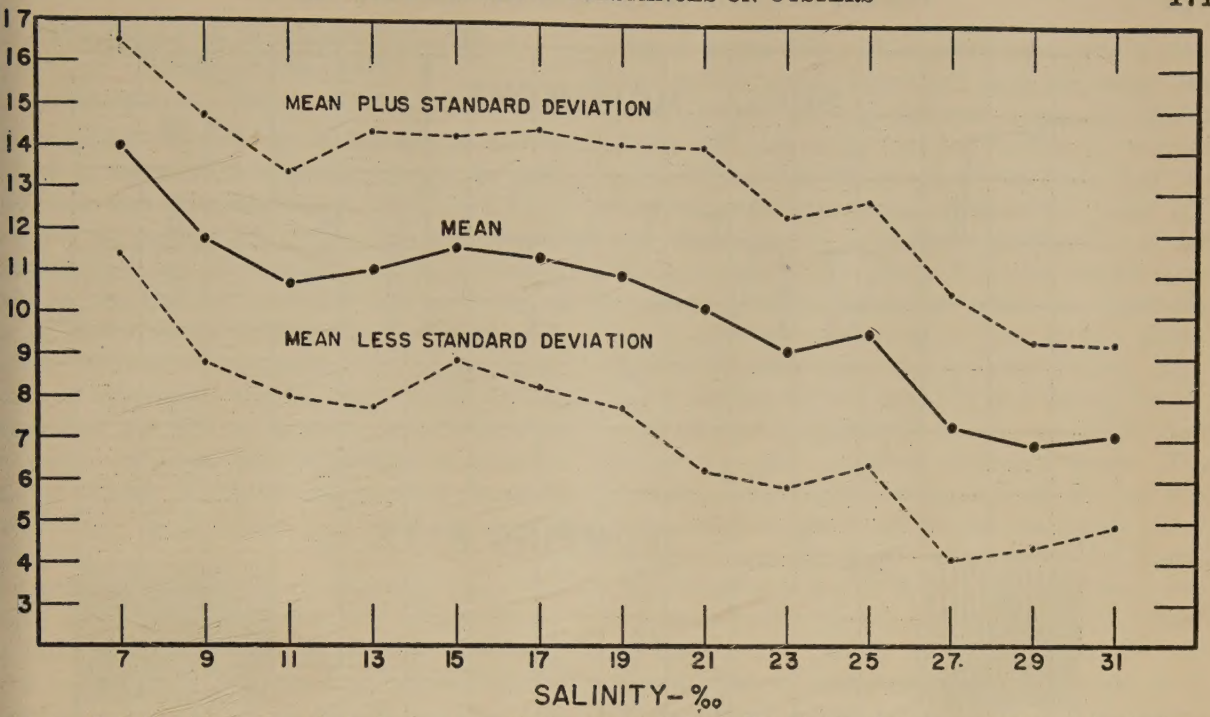
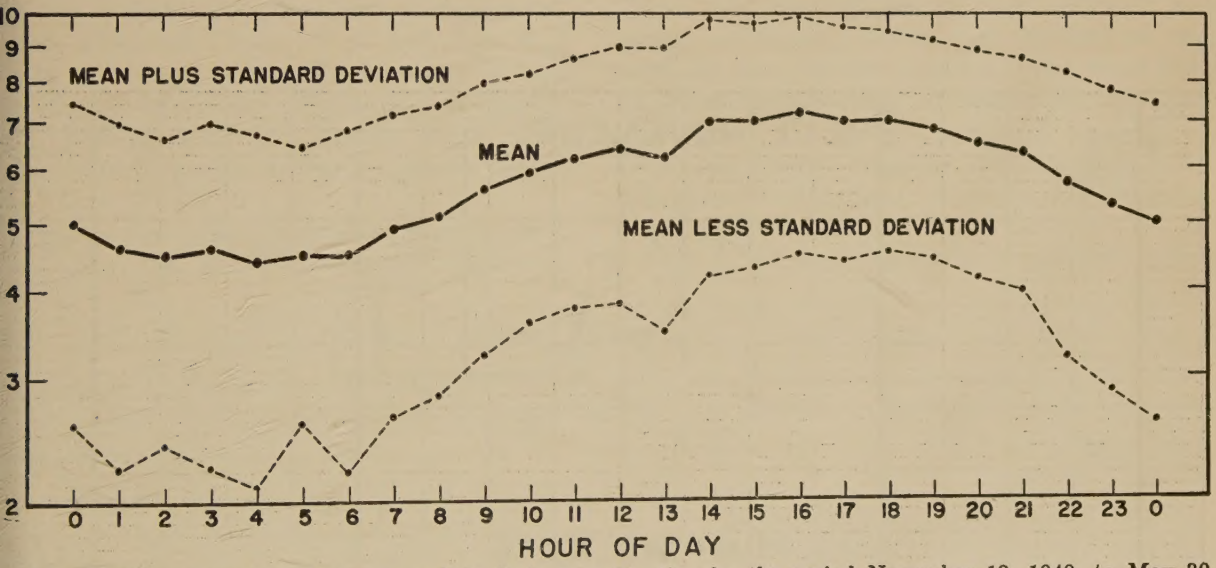


FIGURE 1.—Behavior of carbohydrates in standing sea water. A.—Exposed to daylight, unfiltered, with agita B.—Exposed to daylight, unfiltered, without agitation. C.—Exposed to daylight, filtered, aerated and nonaera D.—Kept in dark, filtered and unfiltered.



URE 2.—Relation between carbohydrate concentration and salinity. The limits of the mean, plus and minus one standard deviation, are shown.



URE 3.—Average diurnal variation in carbohydrate concentration for the period November 13, 1949, to May 30, 1950, plotted on semilogarithmic scale.

DIURNAL VARIATION

distinct diurnal variation became evident
en the logarithms of the carbohydrate concen-
ions were plotted against the hour of day for
period from November 13, 1949, (when the
ervations were increased from bihourly to
rly) until May 30, 1950. This cycle was evi-
t whether the data were averaged monthly or
the entire period. The data of averages for the entire period are shown in figure 3; also plotted are the standard deviations computed at each hour.

From the curve (fig. 3) it is apparent that the concentration reaches a minimum at about 02^h00^m where it remains until about 14^h00^m; it then increases steadily to a maximum at 11^h00^m. This maximum is maintained until 18^h00^m, when the concentration begins a nocturnal decline.

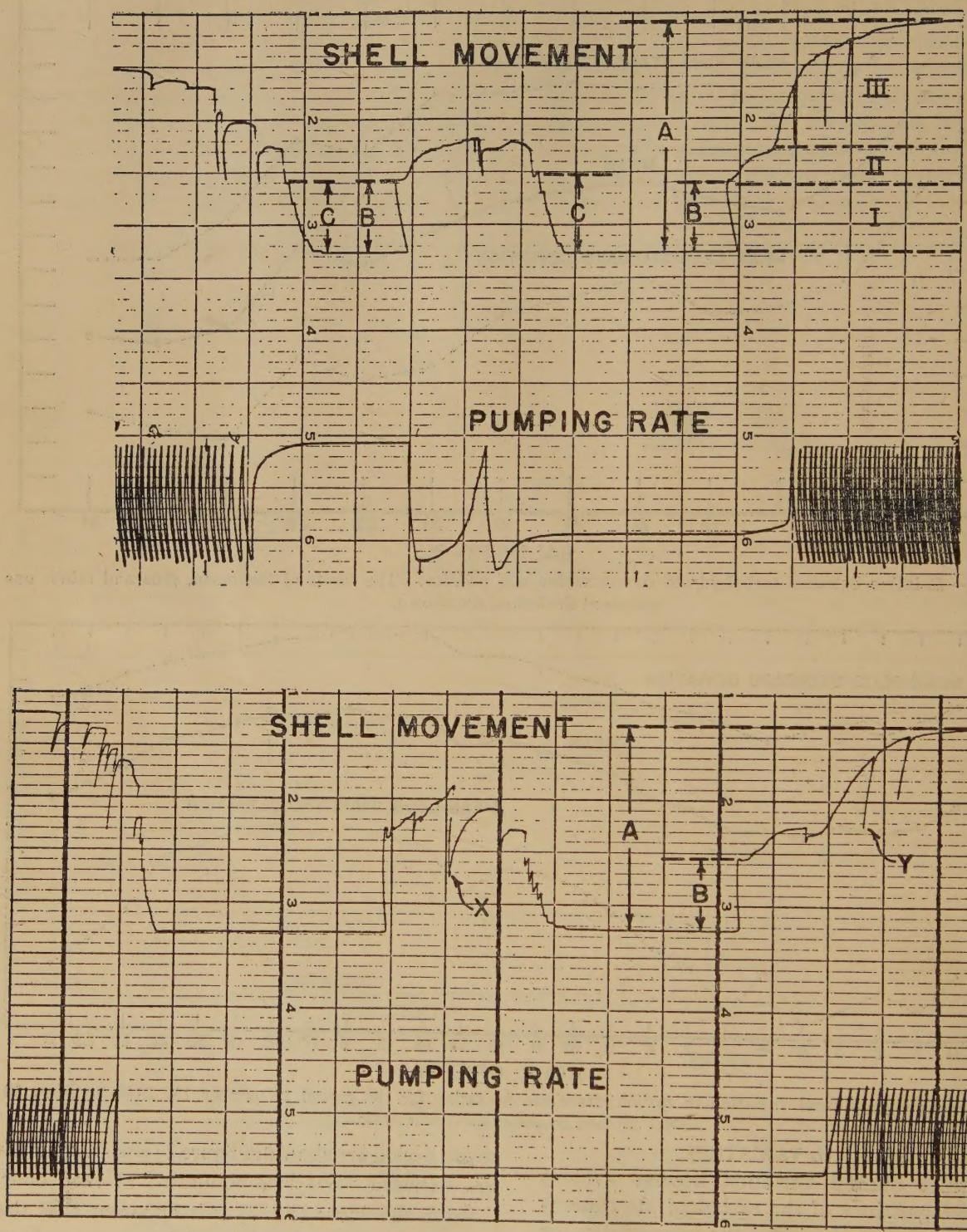


FIGURE 4.—Reproductions of actual records of shell gape and pumping rates, demonstrating the basic component the shell movements of oysters. In each, *A* indicates the full amplitude of gape, *B* the opening movement of phas *C* the closing movement, which is always done in "steps." Lower figure shows two additional types of closu *X* an abnormal one resulting from mechanical disturbance, *Y* a typical snap closure, probably to eliminate unwan substances. Paper speed 2 inches an hour; vertical lines represent quarter-hour intervals.

THE OYSTER'S RESPONSE TO CARBOHYDRATES

ANALYSIS OF SHELL MOVEMENTS

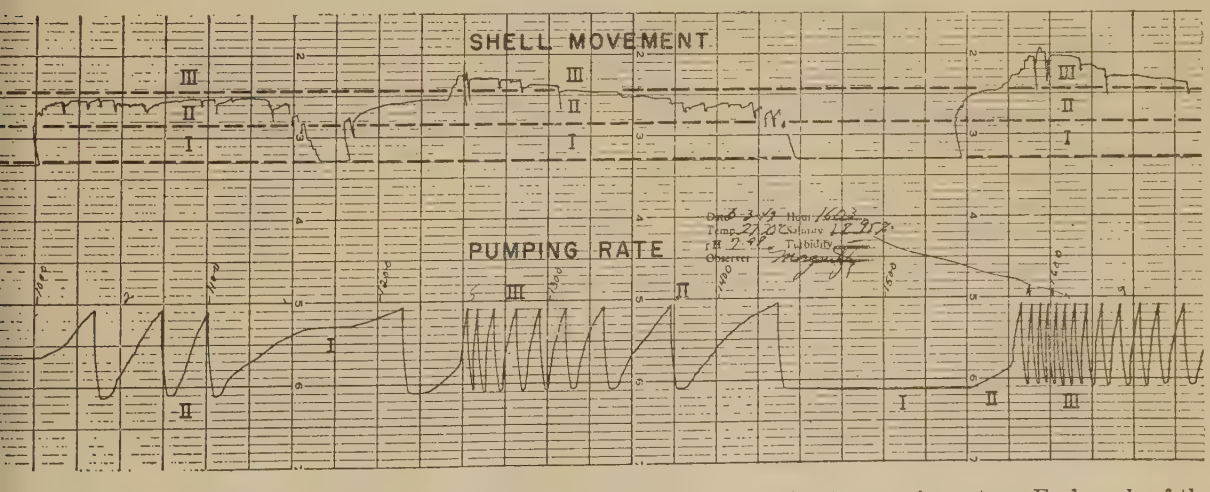
n analyzing the activity of oysters, as shown ier, it is necessary to consider separately shell ements and pumping rates. First, we have mpted to isolate and define the elements of relaxation and contraction of the adductor cle as reflected by recorded shell movements s to relate oyster activity to carbohydrates. igure 4 illustrates representative shell move- nts. *A* represents the full gape of the oyster recorded by the apparatus. This range of enness," or gape, can be divided into three ls, or phases, which we designate as phases I, and III. Each phase has its physiological ificance.

Ve interpret phase I as resulting from the vity of a single, special set of muscle fibers. s characteristic of these fibers that they relax or none," and thus cause the almost instan- eous gape as typified by *B*. Notice that this of fibers does not close the valves with a single ep but in steps which have been designated as ppe" (Galtsoff 1946). It is apparent that re is a distinct mechanism involved in this se of shell movement. This phase is charac- zed, both opening and closing, by a more d response to external stimuli than are phases nd III.

Ve believe that phase II must be regarded as a yed phase III, since it is a transition between phases I and III. This phase represents a testing period, and probably involves only the promyal and cloacal passages, since the oyster rarely will pass more than 6 or 7 liters of water an hour during this phase. It is probable that the musculature involved is the same as in phase III, but that there is a repressor mechanism which delays progress into phase III until certain environmental requirements are satisfied. Normally an oyster will not remain long in this phase. Figure 4 makes clear why we refer to any opening not going beyond phase II as a testing period.

In phase III, an oyster attains maximum gape and pumps the maximum amount of water. The pumping rate varies with conditions, so that it is essential to record both effluent and shell movement. Phase III, then, represents the full degree of valvular gape which under ideal conditions continues except for certain anomalies. This portion of the curve is broken at various intervals with partial closures indicated in figure 4 (lower) as *Y*. These anomalous closures might be termed "expulsion," or "snap," movements of the valves, probably to void accumulations of inert solids or irritating substances. These snap closures do not normally enter the zone of phase II, but when they do, the rate of reopening is much slower than when they do not. In figure 4 (lower), *X* indicates an abnormal closure resulting from a mechanical disturbance to the oyster. Note that the reopening is much slower than from the *Y* closure.

Each of the three phases has associated with it



RE 5.—The basic components of shell movements of oysters and their relation to pumping rates. Each cycle of the measuring box represents a minimum of 500 milliliters; the Roman numerals on the pumping rate curve correspond to the three phases demonstrated. Reproduction of actual record. Paper speed 2 inches an hour; vertical lines represent quarter-hour intervals.

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definite characteristics of flow through the branchial system (here we arbitrarily include the promyal passage). Phase I may permit a slight flow or none at all; phase II involves some flow, although much less than does phase III. The relation between these phases and their respective pumping rates are typified in figure 5, which is a reproduction of an actual record showing rates of pumping in each of the three phases.

EFFECT OF CARBOHYDRATES ON PUMPING RATE

In these experiments the carbohydrate concentration in the laboratory sea-water supply varied widely. Variations over the entire range within a few hours were common; often variations were 100 percent within 2 hours. Such extreme variation precluded the use of averages in analyzing the data relating carbohydrate concentration to pumping rate.

The hourly effluents, therefore, were computed from pumping rates measured at the time the carbohydrate concentrations were measured. Samples for these carbohydrate determinations were taken from the inhalant side of the oyster, not from the sampling wheel (see Appendix, p. 18 which was located at some distance from oyster. Over a long period of time using a num of oysters, we have found a positive correlat between the carbohydrate concentration and pumping rate of the oyster, as shown in figure

An interesting phenomenon, observed in detailed study of the oyster's response to car hydrates, is the testing period, illustrated figure 7. Variation of the carbohydrate c centration at a temperature of approximately C. is illustrated in the upper figure. U 10^h00^m, the carbohydrate concentration was ab 6 mg./l., followed by a rise within 2 hours to 10 mg./l. level. At the beginning of the inter marked *A* the valves opened into phase II, dur which period a small amount of water was pas through the oyster. At this temperature carbohydrate level was too low to stimulate oyster to further activity. After a short per of closure, another test was made at interval Within 30 minutes, the concentration of car hydrates having risen while the valves were s in phase II, progress into phase III was indu as shown in intervals *C* and *D*. Similarly, but

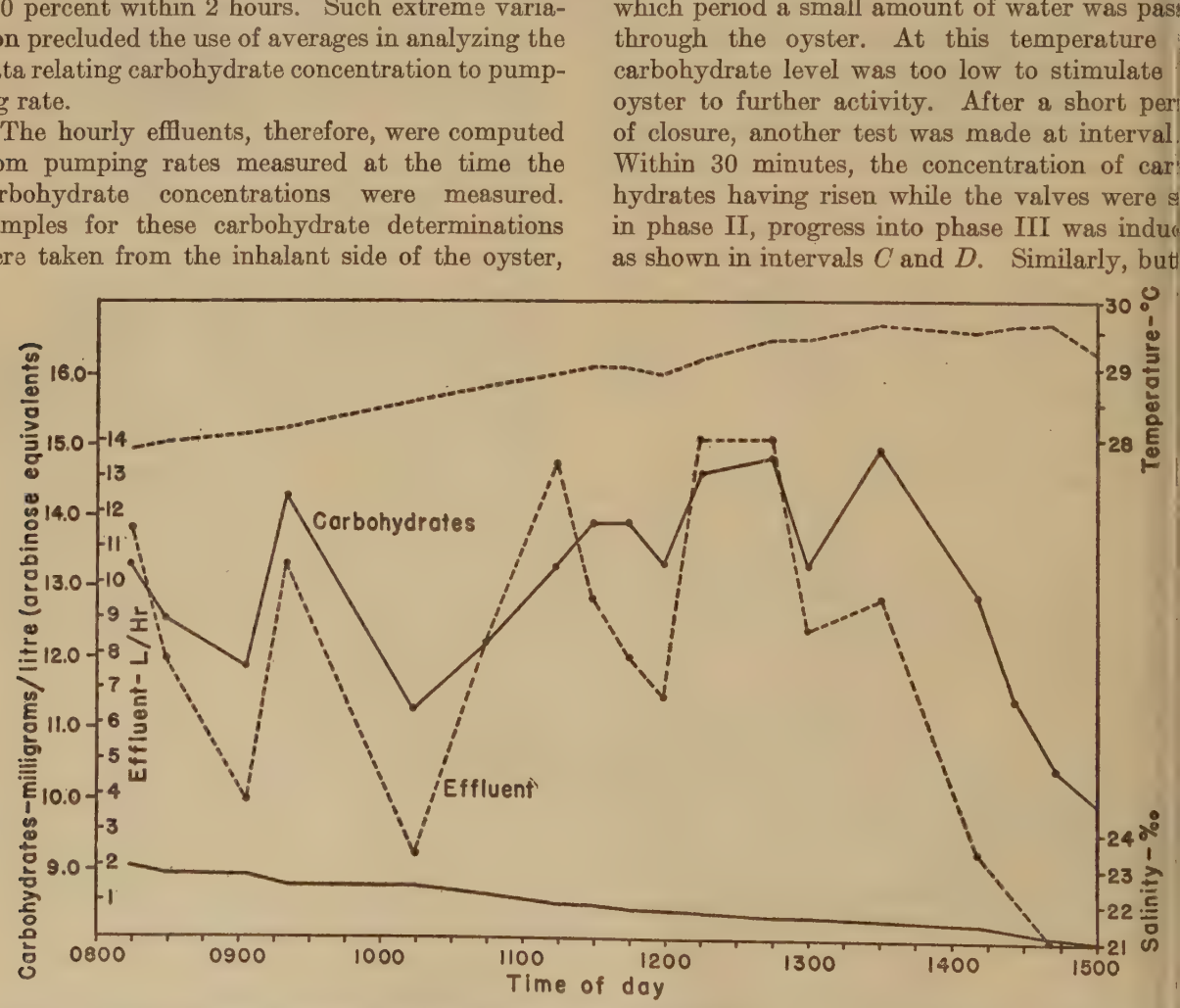
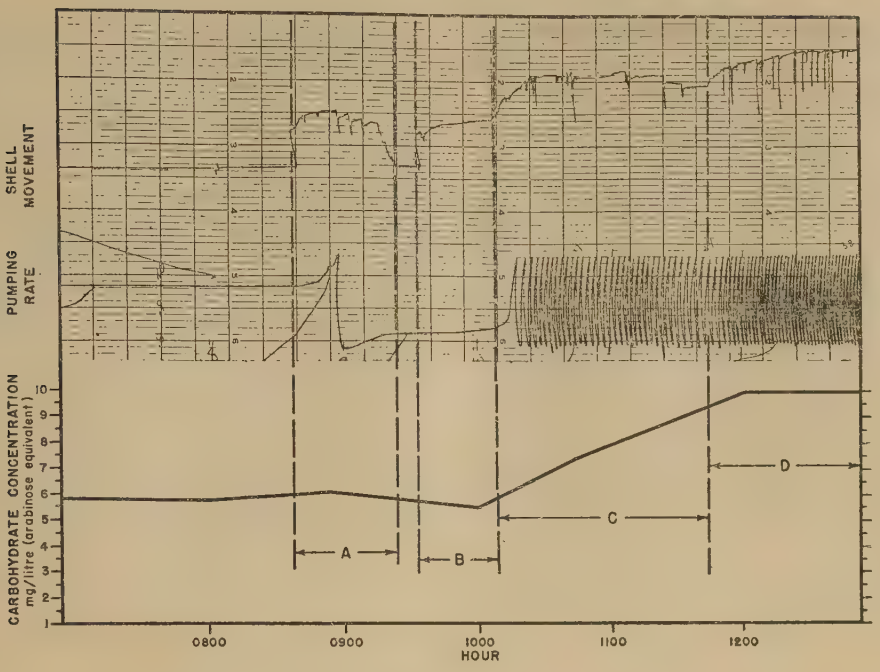
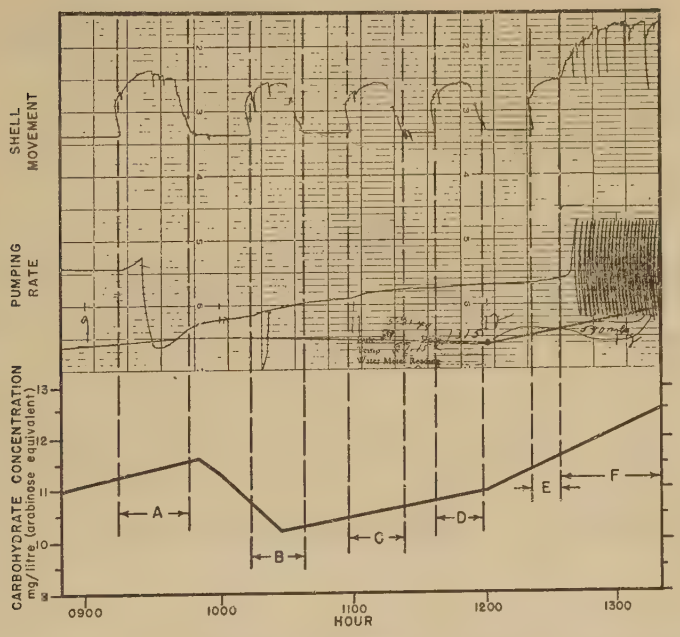


FIGURE 6.—Relation between pumping rates and carbohydrate concentrations. The pumping rates were determined the moment the carbohydrate samples were taken. The mean carbohydrate concentration is 13.4, and the m pumping rate 9.15 liters per hour. The correlation coefficient, 0.78, is significant at the 1-percent level.





URE 7.—Relation between activity of oysters and variations in carbohydrate concentrations at two temperature ranges. Upper figure at temperatures approximating 25° C., lower figure approximating 27° C.; salinity ranges 17° to 20°/₀₀. The carbohydrate threshold for the oyster at 25° C. (upper figure) apparently approximates 6 mg./l. Testing periods are shown as intervals *A* and *B*, phase III as *C* and *D*. Lower figure (27° C.) shows four similar testing periods *A*, *B*, *C*, and *D*; on the fifth test, *E*, the carbohydrate concentration had increased sufficiently to induce phase-III pumping, interval *F*. This oyster, at the higher temperature, apparently had a much higher threshold level (approximating 12 mg./l.) than that of the oyster at 25° C. Reproductions of actual records. Paper speed 2 inches an hour; vertical lines at quarter-hour intervals.

an increased carbohydrate concentration because of increased temperature, the oyster recorded in the lower figure went from phase II into phase III at interval *E*, following four unfavorable testing periods *A* through *D*.

The association of increased pumping rate with a threshold level of carbohydrate concentration and the effect of increasing temperatures on this threshold level are obvious.

Having demonstrated the instantaneous pumping-rate response of the oyster to changing concentrations of carbohydrates, we examined the records of cumulative hourly pumping-rate responses to these changes. Figure 8 showing on a semilog scale the reaction of a typical oyster (No. 92), and of five oysters combined, to the changes in carbohydrate concentration, make evident that despite the stated limitations of method to measuring the carbohydrate activ ratio, the relative changes in hourly effluents parallel very closely the relative changes carbohydrate concentrations. The low efflu rate preceding 18^h00^m on January 27 is coincid with phase-II pumping, and occurs with carbohydrate concentrations below the thresh level for that temperature. With the rise carbohydrate concentration above the thresh the pumping rate increases to that of phase II

Figure 9 illustrates, on a semilog scale, relation of the average-cumulative-daily efflu to average-daily level of carbohydrate concen tion, based on records of two to four oys

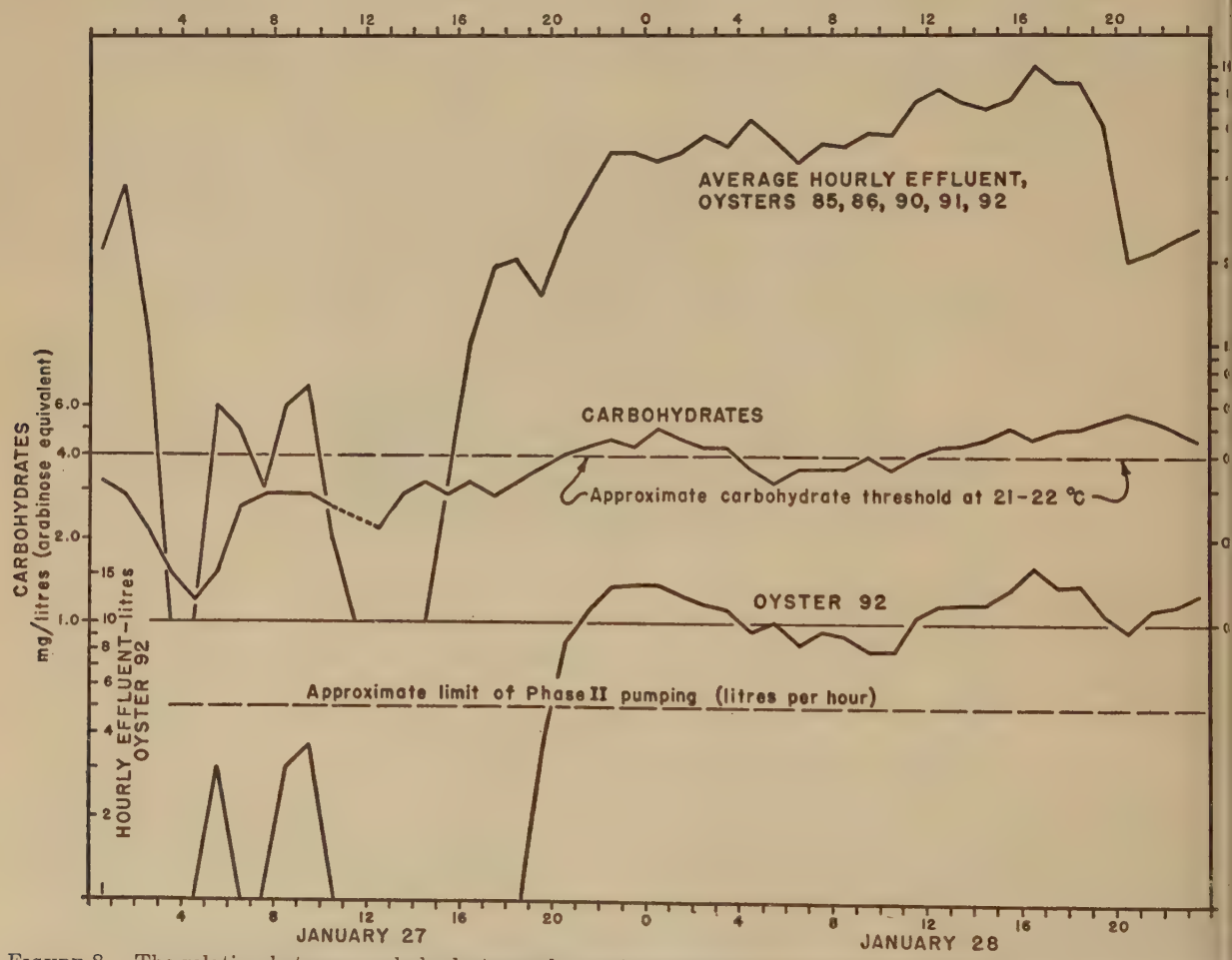

AVERAGE HOURLY EFFLUENT, OYSTERS 85, 86, 90, 91, 92
CARBOHYDRATES
Approximate carbohydrate threshold at 21-22 °C
OYSTER 92
Approximate limit of Phase II pumping (litres per hour)
CARBOHYDRATES mg/litres (arabinose equivalent)
HOURLY EFFLUENT-litres OYSTER 92
JANUARY 27
JANUARY 28

FIGURE 8.—The relation between carbohydrates and cumulative-hourly effluents over a 2-day period, plotted on sem scale. The curve for an individual oyster is shown, together with the average values for all the oysters being reco during the same period. In each case the hourly effluent went above the phase-II level when the carbohydr went above 4 mg./l. During the twentieth hour of January 28, an external factor interfered. The oysters respon but had begun to recover at the close of the period

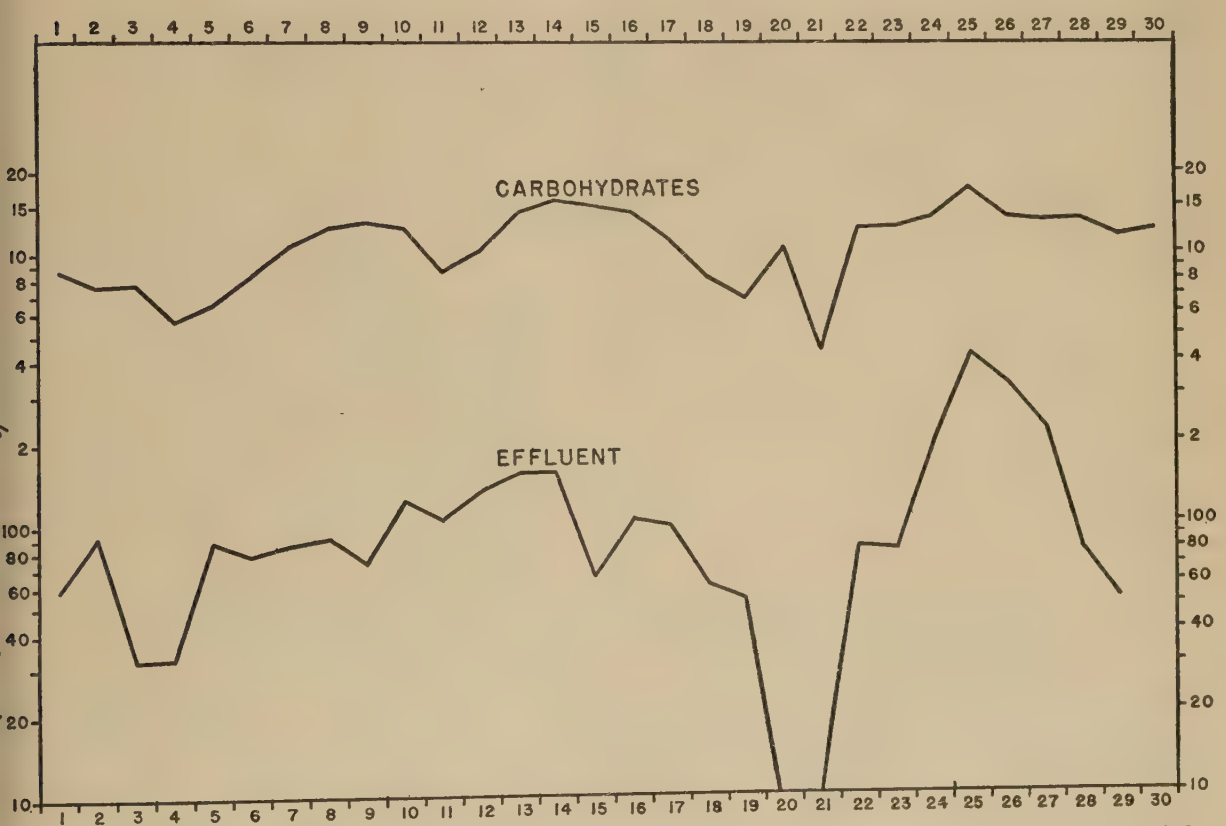
lable through a 30-day period. This com-
ion was made, despite its recognized limitation,
tablish the relation over a long period. It is
e evident from an examination of this figure
such a definite relation does exist.

gures 10 and 11 illustrate the relation that
s between carbohydrates and temperature as
ors influencing the average pumping rate.
ure 10 is based on two defined temperature
ges, while figure 11 is based on a division
rding to season, i. e., the warm months, May
o November 13, and the cold months, Novem-
13 to January 31, of the northern coast of the
of Mexico.

is apparent that water temperature above
C. are unfavorable for oysters in regions in which the production of the dissolved carbohydrates is low.

ASSIMILATION OF CARBOHYDRATES

The oysters can and do remove variable quantities of carbohydrates from the water. This has been ascertained by determining the amount of carbohydrate in the water before it enters the valves of the oyster and after it has passed through the body. The quantities removed do not seem to be related to any of the other measurable activities of the oyster, and, of course, the results could be affected by the oyster's waste metabolites, which are present in the exhalant water. The data of table 2 indicate that up to 50 mg./hour are removed. This is a significant quantity of such material, and assuming that all is utilized,



URE 9.—Relation between carbohydrates and average-cumulative-daily effluents of two to four oysters over a 30-day period, June 1–30, 1949, plotted on a semilog scale. While data from four oysters were combined in establishing this curve, mechanical difficulties reduced the number to two during part of the time.

it represents an oxygen consumption [3] of approximately 50 mg./hour. Yonge (1928), in performing experiments on the mode of utilization of dissolved glucose, found that European oysters (*Ostrea edulis*) removed approximately 20 mg./hour. He felt that the removal took place in the stomach, and earlier (1926) had concluded that there was no evidence of any enzymes free in the gill mucus. This latter would not be necessary if the substance in question were adsorbed on the mucous train and carried into the alimentary tract.

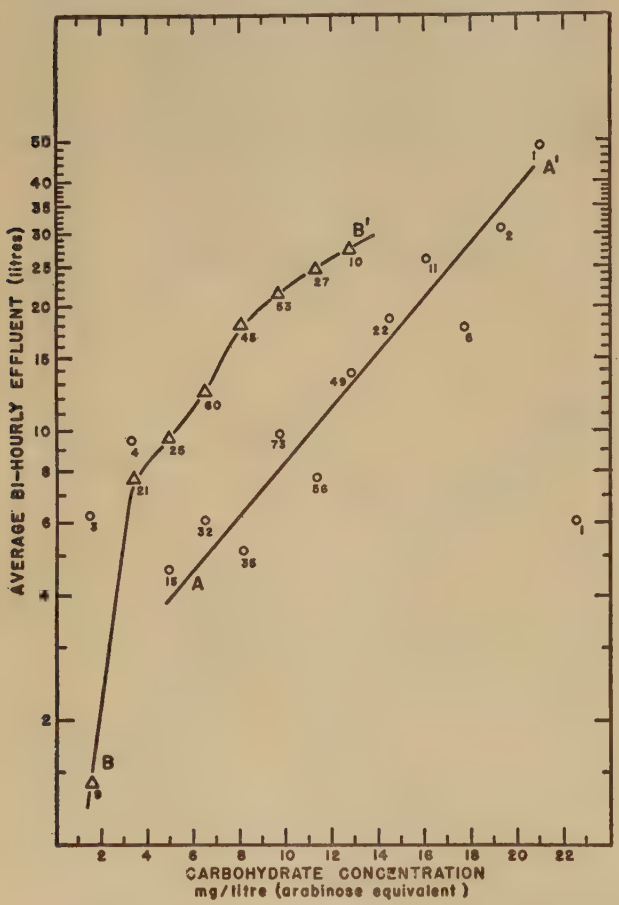


FIGURE 10.—The shift in the carbohydrate-pumpage relation due to temperature. The values are derived from the simultaneous observation of temperature, carbohydrate concentration, and bihourly effluent. Curve *A-A'* is for the temperature range 25° to 30° C., inclusive; curve *B-B'* is for 14° to 21° C., inclusive. The temperatures shifted from one range to the other so quickly that there were insufficient frequencies for analysis in the 22.0° to 24.9° C. bracket. The small figures at each point represent the number of samples from which the value is derived.

[3] According to our later studies (Collier, Ray, and Magnitzky, manuscript in preparation), the oyster actually does utilize oxygen on a scale commensurate with this figure.

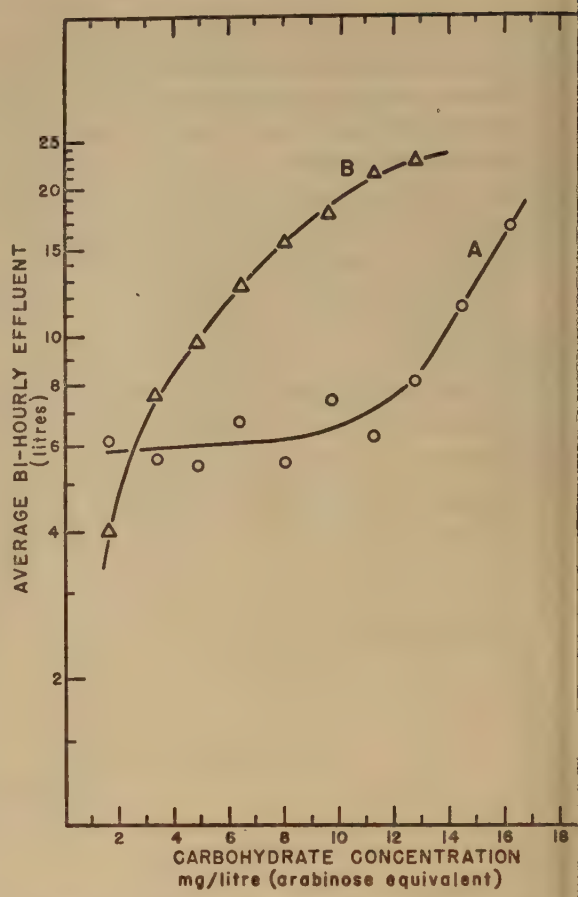


FIGURE 11.—Relation between carbohydrate concentration and the bihourly effluent of oysters. Curve derived from 3,891 bihourly observations on 12 oys from May 10, 1949, to November 13, 1949. The portion of the curve represents the dominance of ph II pumping (testing periods) at carbohydrate value less than 12 mg./l. during the warm months. Curv is derived from 5,464 hourly observations on 11 oys for a period of winter temperatures, November 1949, to January 31, 1950. Semilog coordinates.

TABLE 2.—*Removal of carbohydrates from sea water oyster 74, May 24–June 11, 1949*

Pumping rate [1]	Temperature	Concentration of carbohydrates in sea water	Hou rate of bohyd remo
L./hr.	*° C.*	*Mg./l.*	*M*
12.7	26.1	12.1	
13.9	25.9	12.1	
17.1	26.7	7.3	
17.6	25.9	10.5	
24.4	28.4	6.6	
24.6	25.9	11.2	
25.2	27.2	8.7	
26.2	27.2	6.3	
26.4	28.8	11.0	
27.9	28.5	9.1	
29.3	28.0	8.2	
33.4	27.5	10.5	

[1] Some of these values were obtained while the oyster was going from I to phase II; therefore, the pumping rates shown do not necessarily r the correlation of pumping rate and carbohydrate concentration.

ANOMALOUS RESPONSES

ne carbohydrate-temperature relation is not only factor to which oysters respond. Others involved, and they must often dominate the er's behavior pattern, and conceal the effects he carbohydrates. As previously stated, it t be recognized that we do not know that the tances indicated as carbohydrates by the test always true carbohydrates. Further, we can- say which of the many carbohydrates are onding to the test, nor can we say which of carbohydrates are represented. There is the ibility that the oyster is responding to a single ohydrate which varies considerably, but whose ation may be completely hidden by other ohydrates which may be far more abundant imes. Bell (1948) states that "numerous so-called glycogens in plants and animals may not be chemically identical with animal glycogens, which may quite well vary among themselves." Since glycogen is one of the carbohydrates, the significance is apparent.

Figure 12 demonstrates an extreme of variation between oysters in their responses to variations in carbohydrate concentrations. Note that oyster 88 was comparatively insensitive to carbohydrate changes during the first few days, especially on December 7. By comparison, oyster 87 was markedly responsive to the material throughout. Despite the anomalies (which may be due to the sampling difficulties previously pointed out), these figures illustrate the influence of consistently low carbohydrate levels, particularly during the winter period.

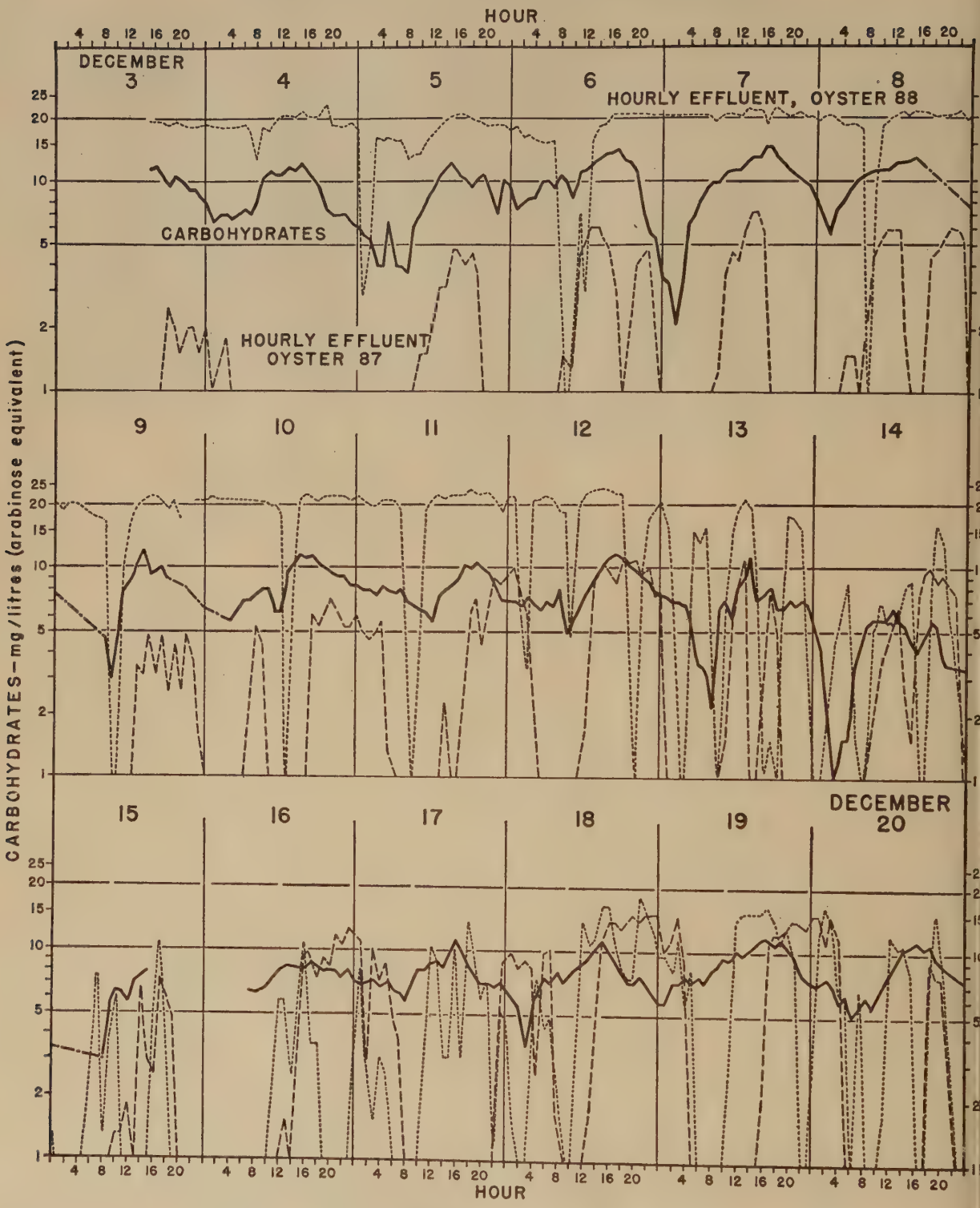


FIGURE 12.—Example of extremes in pumping-rate response of two oysters to a common carbohydrate concentra over an 18-day period plotted on a semilog scale. It is likely that oyster 88 had a glycogen reserve at the st so was not as dependent on external nutrients as was 87.

SUMMARY

uring prolonged and detailed studies of the ts of industrial wastes on oysters, *Crassostrea nica* (Gmelin) at Pensacola, Fla., we noted shell gapes and pumping rates of oysters r simultaneous observation behaved in a llel manner as though responding to a com- factor. It was found that this behavior ern was related to the concentration of cer- organic substances dissolved in sea water, that these substances responded to the hyl-carbazole test for carbohydrates.

ne simultaneous recording of shell movements pumping rates was chosen as the best avail- index of physiological activity for establish- this relation, since both lend themselves to terrupted recording over long periods of . In analyzing these recordings, it became ent that shell movements and pumping rates divided into three distinct levels, which we defined as phases I, II, and III, each with its iological significance. In phase I, there is almost instantaneous gape on opening to t one-third of the maximum gape, a less l step closure, no pumping, and a rapid onse to external stimuli. Phase II, the middle of the total gape, must be regarded as a sition between phases I and III, represents sting period, and probably involves only the ayal and cloacal passages, since the oyster y passes more than 7 liters of water an hour ng this phase. In phase III, the oyster at- maximum gape and pumps the maximum unt of water. Because the pumping rate s with conditions, it is essential to record effluent and shell movement in interpreting effects of varying concentrations of the dis- d carbohydrates. Certain anomalies within phases were noted, including snap closures in phase III. These closures usually do not phase II, but when they do, the rate of ening is retarded.

association between carbohydrate concen- on and salinity was noted, but the signifi- e of this relation remains obscure. It could ne result of physical dilution of carbohydrate- low-salinity inshore water with carbohydrate- high-salinity gulf water, or of the adverse t of high-salinity water on the growth of the nisms creating the carbohydrates.

was established that the oysters remove variable quantities (up to 50 mg./hour) of the carbohydrates from sea water.

Considerable variation in the response of individual oysters to the carbohydrates were noted. This would be expected, since the carbohydrate/temperature factor is not the only one to which oysters respond, and these other factors must often dominate the oyster's behavior pattern, concealing the effects of the carbohydrates. Then, too, the substances which respond to the N-ethyl-carbazole test may not all be true carbodrates, or the oyster may be responding to only certain of the carbohydrates within the carbohydrate complex, whereas the test responds to all.

The concentration of dissolved carbohydrates was found to vary widely in the sea-water supply. Because of this, the relation of carbohydrate concentration to pumping rate becomes obscure unless the pumping rate and carbohydrate concentration were measured simultaneously. A definite response of the oyster in the phase of opening and in the rate of pumping to the carbohydrate concentration was noted. Each oyster appears to have a threshold limit to the carbohydrates below which it will not pump. This threshold is raised with increasing temperatures. A correlation of 0.78, significant at the 1-percent level, was established over a long period of time using a number of oysters when the pumping rate and carbohydrate concentrations were measured simultaneously. Even when average-cumulative-daily effluents and average-daily-carbohydrate levels were compared, the relation between the two was striking. Because of the raising of the threshold level with increasing temperatures, it is apparent that water temperatures above 25° C. are unfavorable for oysters in regions in which the carbohydrate concentration is low.

During the course of these observations certain characteristics of these carbohydrates were investigated. It was established that no significant changes in concentration took place when held overnight either in the refrigerator or at room temperature; that the concentration first increased, and later decreased when exposed to daylight for prolonged periods; that this increase was affected by aeration; that, when filtered or centrifuged, or when held in the dark, no significant change in concentration took place. From these findings it became evident that the carbohydrates result from biotic activity.

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APPENDIX

ESTIMATING CARBOHYDRATES IN SEA WA

The test for carbohydrates was developed Dische (1927) with adaptations to suit our quirements by Erdman, who found that N-et carbazole ($C_{12}H_8NC_2H_5$) was better suited for w with sea water than carbazole (Erdman and L 1950). Further modifications were made for particular type of electrophotometer (Fisher used. All photometer readings were made a green filter which bracketed the range of peak absorptions of the dyes resulting from use of the N-ethyl-carbazole reagent.

The reagent was prepared by dissolving mg. of N-ethyl-carbazole in 250 ml. of prech 90-percent sulfuric acid (reagent grade [1]). It made up in quantities to last not more than hours and stored in the refrigerator. Use of tilled tapwater sometimes resulted in the deve ment of a green color in the reagent, but disti rainwater eliminated this difficulty. The water was Mississippi River water from a s sedimentation and chlorinating plant. No planation is offered for this reaction, but i mentioned as a precaution to any who m apply the test. All glassware, including reag bottles, should be thoroughly seasoned in furic acid before being used.

Exposed to direct sunlight, the reagent turn green and become valueless in approxima 5 minutes. It should be mixed in subdued li and stored in the dark.

In many cases where there is no carbohyd present in the water the reaction will deve into deep green. In deep ocean waters this c has been found to be associated with high nit values; but if carbohydrates are present with nitrates, the deep green will not develop.

The routine procedure was as follows: (1) seawater sample was centrifuged for 10 min at a relative centrifugal force of 336. (2) 2.5-ml. sample was drawn by pipette from top of the centrifuged sample and put in a 25- tube with 22.5 ml. of N-ethyl-carbazole reag and hydrolyzed for 15 minutes at 70° C. ±0 C. A small chip of lint- and paper-free para was dropped on the surface to exclude oxyg (3) After hydrolysis, the sample was cooled

[1] Acid supplied in bottles with seals of cellulose derivatives was co inated by them and was made unsuitable for this test.

to 25 minutes and transferred to a 23-ml. ette. The color density was measured and rded as $-\text{Log } T$.

he densities thus determined were converted quivalent arabinose in milligrams-per-liter by use of a graph constructed from standard dilu- s of l-arabinose. The general precautions aining to colorimetry were observed through- The data for a set of standards are pre- ted in table A–1. The l-arabinose was checked adsorption of atmospheric moisture by a series veighings made over a period of 20 minutes at inute intervals. No increase in weight was ectable on the analytical balance.

BLE A–1.—($-\text{Log } T$) *values for various concentrations of l-arabinose*

Test	1.03 mg. l-arabinose per liter	10.30 mg. l-arabinose per liter	20.60 mg. l-arabinose per liter	51.50 l-arabinose per liter
	2.5	15.5	30.0	74.0
	2.5	15.5	31.0	74.5
	2.0	15.0	30.0	74.0
	2.5	16.0	30.5	74.0
	2.0	15.5	30.0	74.0
	2.0	15.5	30.0	73.5
	2.5	15.0	30.0	74.0
	2.5	15.5	29.5	74.0
	2.0	16.0	30.0	74.0
0	2.0	15.5	30.0	74.0

MEASURING OYSTER ACTIVITY

The principle of the rubber apron originated by ore (1908), and the constant-level chamber eloped by Galtsoff (1926) were combined in study to improve the accuracy of measure- nts of the pumping rate of oysters reported by son (1936).

We found the attachment of the dental dam to valves of the oyster was done most expediently h a small soldering nail and sticks of beeswax. e soldering nail was connected to a suitable ostat and the voltage set to keep the soldering just at, or slightly over, the melting tempera- e of the beeswax. The beeswax was worked small pencils and applied to the shell of the ter with the point of the soldering nail. First, wax was applied along the line of attachment he rubber to fill the irregularities of the shell ch could cause leaks. After this, a small wall vax was built up and the rubber sealed to it. he hinges and in the region of the palliobrachial fusion, small pads of pyrex wool (instead of cotton) were used as packing between the rubber membrane and the shell. The glass does not lose its resilience when wet, neither is it subject to organic decay on long exposure to experimental conditions. The oysters were held in position on Hopkin's stands (fig. A–1) and connected to the trough. To determine the amount of effluent, we used a simple box with an automatic siphon in the place of the usual dumping bucket (Collier and Ray 1948).

We acknowledge the great assistance of Drs. W. E. Hanson and J. G. Erdman of the Mellon Institute of Industrial Research, and we are grateful to W. K. Bowman, of the Gulf Research and Development Company, for providing us with excellent electric kymographs built to the specifications of our project. Some of the early records were made with a paper speed of 2 inches an hour, but this was later changed to 4 inches an hour. At the latter speed, we obtained 2 weeks of uninterrupted recording with superior amplification of detail.

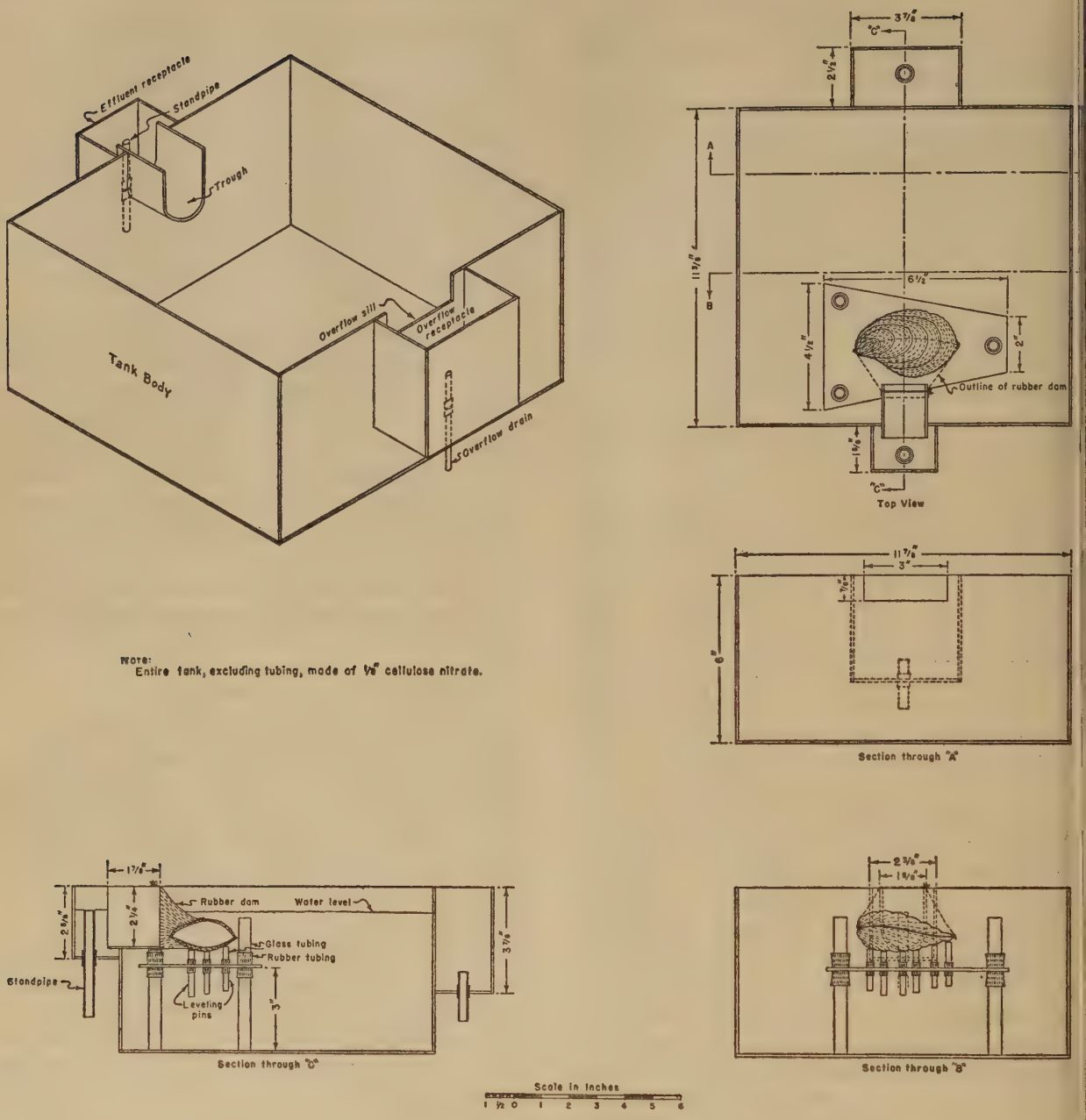
DESCRIPTION OF SAMPLING DEVICE

The sampling device (fig. A–2) consisted of a wheel (A) whose circumference revolved under a continuous stream of water (B), and thus caused the equally spaced tubes (C) to be filled. The interval of filling was regulated by the spacing of the tubes and the velocity of the wheel. The wheel was driven by a synchronous motor (D) whose velocity was 1 revolution in 24 hours.

The wheel was fastened to a vertical shaft by means of a flange; the shaft was suspended at (E) by means of a thrust bearing and set collar, and was connected to the drive shaft of the motor by a tubular coupling. In this manner, exposure of the motor and bearing suspension to salt water was minimized.

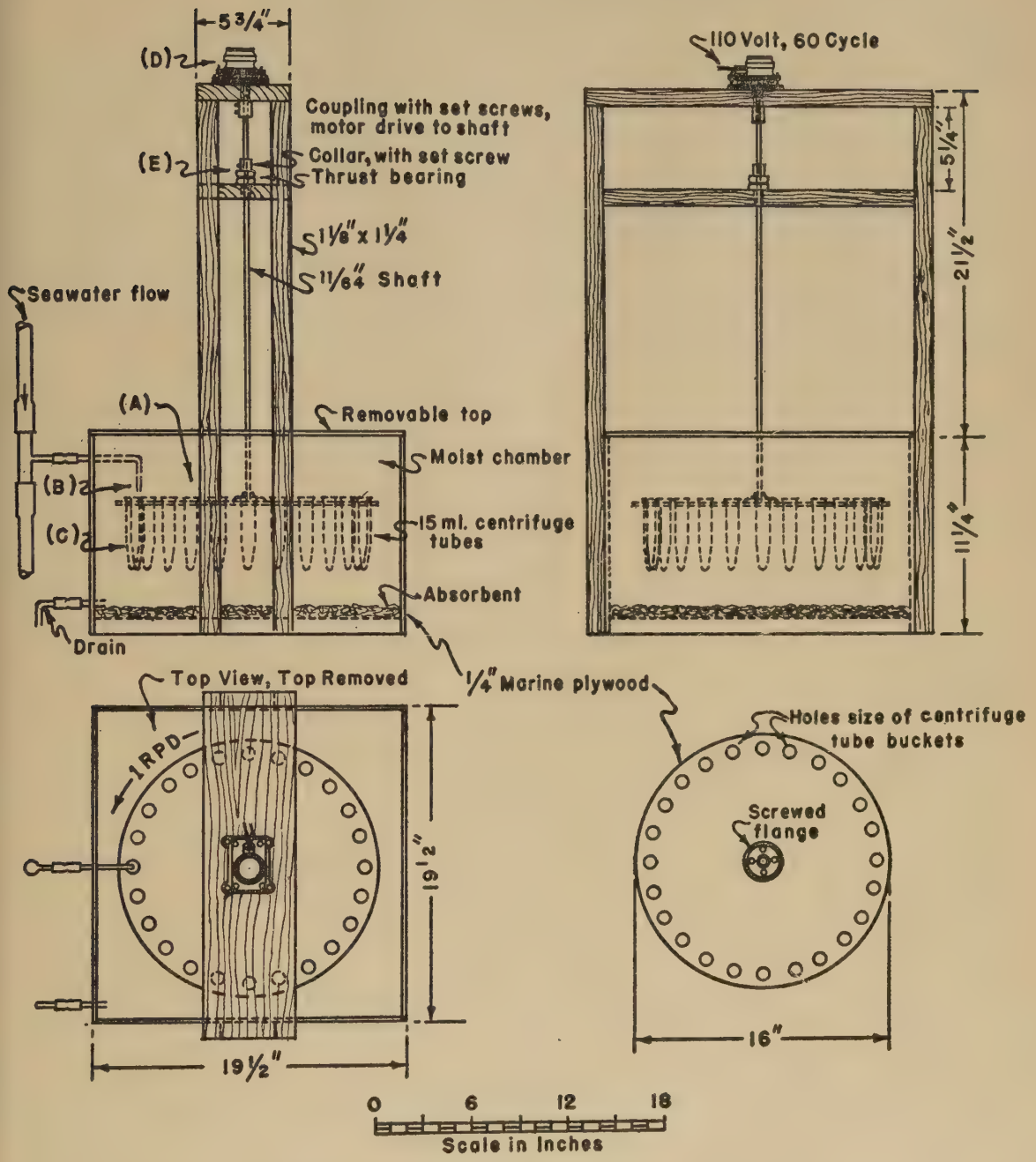
The waste water overflowed onto absorbent material, and this, in combination with the tight lid, kept the atmosphere within the chamber saturated. Evaporation was not sufficient to affect the accuracy required ($\pm 0.05‰$) for salinity determinations.

The apparatus as used by us gave a 15-minute composite sample every 1 or every 2 hours, as desired.



EXPERIMENTAL TANK USED IN MEASURING PUMPING RATE OF OYSTERS

FIGURE A–1.—Tank used in the experimental study of oysters. The oyster is fastened to the Hopkin's stand with beesw at the ends of the small glass tubes. The water level at the top of the standpipe is finely adjusted so as to break o with the addition of two or three drops of water, yet not back the water into the tank body. The tube is cut wit carborundum saw and, once set up, it is never allowed to become dry, because the wetting property of the bacte slime which accumulates there would be destroyed and the "sensitivity" of the tube lost.



URE A–2.—Device for automatically sampling water at hourly intervals. Operated by a synchronous motor, it was so designed as to rotate one of the 24 tubes under the inflow pipe each hour.

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